"FORM PTO-1390 (REV 11-2000)

U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. § 371

ATTORNEY'S DOCKET NUMBER: 303212000600

U.S. APPLICATION NO. (If known, see 37 CFR 1.5): 09/980717

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB00/01736	05/05/2000	07/05/1999

TITLE OF INVENTION

A COMPOUND FOR USE IN MEDICINE

APPLICANT(S) FOR DO/EO/US

James Anthony GALLAGHER; Wayne Barry BOWLER; Simon Christopher WAGSTAFF

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application under PCT Article 19 (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information: return receipt postcard.

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sd-64822

U.S. APPLICATION NO. (if known, see 37 CFR 1.5) * 09/980717	INTERNATIONAL APPLICATION NO. PCT/GB00/01736	ATTORNEY'S DOCKET NUMBER: 303212000600

21. ☒ The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO.....$1,000.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO.....$890.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....$710.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provision of PCT Article 33(1)-(4) $690.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00

	CALCULATIONS PTO USE ONLY	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$890.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$130.00	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	20- 20 =	0	x $18.00	$	
Independent claims	4 - 3 =	1	x $80.00	$80.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	$*	

TOTAL OF ABOVE CALCULATIONS =	$	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by ½.	$	
SUBTOTAL =	$	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +	$*	
TOTAL NATIONAL FEE =	$*	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00 per property** +	$*	
TOTAL FEES ENCLOSED =	$1100.00	
	Amount to be refunded:	**$***
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a. ☐ A check in the amount of $* to cover the above fees is enclosed.

b. ☒ Please charge my **Deposit Account No. 03-1952** in the amount of $1100.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees that may be required, or credit any overpayment to **Deposit Account No. 03-1952**. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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PATENT

Docket No. 303212000600

CERTIFICATE OF MAILING BY "FIRST CLASS MAIL"

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Tami M. Procopio

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

James GALLAGHER, *et al.*

Serial No.: To be assigned

Filing Date: Even date herewith

For: A COMPOUND FOR USE IN MEDICINE

Examiner: To be assigned

Group Art Unit: To be assigned

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to examination of the above-referenced application, please amend the claims as follows:

AMENDMENT

In the Claims:

Please cancel claims 1-20 and substitute the following claims:

21. (New) A method to identify a compound which inhibits the expression or function of an ABC protein in bone, which method comprises

determining the ability of said compound to inhibit bone resorption in a subject, whereby a compound that inhibits bone resorption is identified as a compound which inhibits expression or function of ABC protein in bone.

22. (New) A method to identify a compound which inhibits bone resorption, which method comprises

determining the ability of the compound to inhibit the expression or function of an ABC protein in bone, whereby a compound that inhibits the expression or function of an ABC protein in bone, identified as a compound which inhibits bone resorption.

23. (New) A method to treat conditions benefited by inhibition of bone resorption which method comprises administering to a subject in need of such treatment an amount of an inhibitor of ABC protein function or expression effective to result in inhibiting said bone resorption.

24. (New) The method of claim 23, wherein said compound is selected from the group consisting of glibenclamide, tolbutamide, chlorpropamide, tolozamide, glipizide, gliquidone and gliclazide.

25. (New) The method of claim 23, wherein said administering is oral or intravenous.

26. (New) The method of claim 23, wherein said amount is 14 μg - 71.5 mg/kg body weight per day.

27. (New) The method of claim 26, wherein said amount is 140 μg - 7.15 mg/kg body weight per day.

28. (New) The method of claim 23, wherein said condition is osteoporosis.

29. (New) The method of claim 23, which further comprises administering parathyroid hormone.

30. (New) The method of claim 29, wherein said parathyroid hormone is administered by oral, subcutaneous or intravenous administration.

31. (New) The method of claim 29, wherein parathyroid hormone is administered in an amount of 0.014-2.9 IU/kg of body weight.

32. (New) The method of claim 31, wherein the parathyroid hormone is administered in an amount of 0.7-1.4 IU/kg of body weight.

33. (New) A pharmaceutical composition for inhibition of bone resorption in a subject in need of such treatment which composition comprises an effective amount of a compound which inhibits the function or expression of ABC protein in bone.

34. (New) The composition of claim 33, wherein said compound is selected from the group consisting of glibenclamide, tolbutamide, chlorpropamide, tolozamide, glipizide, gliquidone and gliclazide.

35. (New) The method of claim 34, wherein said compound is present in unit dosage form.

36. (New) The composition of claim 35, wherein said compound is present in an amount of 1 mg - 5 g.

37. (New) The composition of claim 36, wherein the compound is present in an amount of 10 mg - 0.5 g.

38 (New) The composition of claim 33, which further contains parathyroid hormone.

39. (New) The composition of claim 38, wherein said parathyroid hormone is present in an amount of 1-2,000 IU.

40. (New) The composition of claim 39, wherein said parathyroid hormone is present in the amount of 50-100 IU.

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sd-65331

REMARKS

The claims have been reworded and amended to conform to U.S. practice. It will be noted that claims 1-16 as published in the PCT application are directed to a "second medical use form" as used in non-U.S. Patent Offices. Currently proposed claims 23-40 are directed to methods of treatment of an alternative form of this second medical indication in conformance with practice in the U.S. Claims 21 and 22 correspond to claims 17 and 18 in the PCT publication. No new matter has been added and entry of the amendment is respectfully requested.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket No. 303212000600.

Respectfully submitted,

Dated: November 7, 2001 By: Kate H. Murashige

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DESCRIPTION

A COMPOUND FOR USE IN MEDICINE

The present invention relates to the use of a compound and composition which will act as an inhibitor or antagonist of the expression or function of an ABC protein in bone, more particularly an osteoclast associated ABC protein, for use in medicine, and more particularly to the use of a compound and composition which will act as an inhibitor or antagonist of the expression or function of an ABC protein, more particularly an osteoclast associated ABC protein for use in the manufacture of a medicament for use in the treatment of a disease where full or partial inhibition of bone resorption will result in an improvement in the disease. Such diseases include, but are not limited to, osteopenia, such as osteoporosis, Paget's disease, bone metastases, myeloma, periodontal disease and humoral hypercalcaemia of malignancy.

The invention also relates to a method of screening for a compound which will act as an inhibitor or antagonist of the expression or function of an ABC protein in bone, more particularly an osteoclast associated ABC protein, comprising determining whether the presence of said compound leads to full or partial inhibition of bone resorption.

ABC proteins (ATP binding cassette proteins), also called traffic ATP ases, are a super family of transmembrane proteins involved in the movement of substrates across cell membranes. ABC proteins are abundant in prokaryotes where they

represent almost 5% of the total genome and show specificity for a diverse range of substrates ranging from peptides and amino acids to ions and sugars. ABC proteins are characterised by the presence of 2 peptide motifs, Walker A and Walker B motifs. These motifs are common to many nucleotide binding proteins. However, ABC proteins are distinguished from these other proteins by the presence of a third C-signature motif, separating Walker A and B motifs with conserved spacing.

The importance of ABC proteins in mammalian systems is now being recognised. Several members of the ABC family have been shown to be important in human disease, their dysfunction results in a variety of disease states including cystic fibrosis, multi-drug resistance of tumour cells, non-insulin dependent diabetes and adrenoleukodystrophy. These ABC proteins are known to be involved in the translocation of ion and hydrophobic drugs across the plasma membrane. Other human ABC proteins have also been shown to be involved in peptide translocation (PAB) and phospholipid transfer across the canalicular membrane, as is the case with the MRP sub family. In addition, human ABC - 1 has recently been implicated as a regulator of phospholipid equilibrium and non-classical (signal independent) secretion of IL1- β in macrophages.

The applicant has discovered ABC transporter proteins in bone and osteoclast rich tissue and identified several novel members of the ABC protein family from osteoclastoma cDNA libraries and human bones cDNA libraries by immuno-screening and hybridrisation screening.

The applicant's discovery has indicated that compounds which will either

inhibit or promote expression or function of an ABC protein, more particularly an osteoclast associated ABC protein, may be useful in treating conditions arising out of osteoclastic function.

This role of ABC proteins, more particularly an osteoclast specific protein has not previously been identified.

However, in view of the fact that P-glycoprotein has recently been found to be present in osteoblasts (Calcified Tissue International, 1996 Vol 58 No. 3 P186-191) one can postulate that members of the ABC family of proteins may be involved in bone formation.

More generally, ABC cassette proteins have been implicated in many cellular functions. As such an osteoclast associated family of ABC transporters may regulate many processes within these cells.

The ability of members of the ABC super family to regulate volume-activated channels via ATP release has been documented in other cell types.

Osteoclasts are terminally differentiated cells and are hence programmed to die by apoptosis. The ABC1 member of the ABC superfamily has been implicated in the recognition of apoptotic cells by macrophages, a process thought to involve transmembrane flux of phosphatidylserine.

Inhibition or promotion of some of these putative functions of ABC proteins expressed in bone and osteoclasts is indicated as having complex effects on, for example, bone resorption ranging from inhibition, through no effect, to stimulation. Inhibition of osteoclast apoptosis, would lead to a subsequent elevation in the

functional osteoclast pool and enhanced resorption. Similarly, sulphonylurea sensitivity is conferred on K/ATP channels through the presence of ABC transporters, inhibition of which blocks potassium ion efflux and consequent calcium ion influx thereby promoting insulin secretion. It is therefore indicated that blocking osteoclast associated ABC transporters associated with ion channels will enhance release of factors that may stimulate resorption, including regulatory factors, protons and proteases including Cathepsin K. Conversely, the processes of osteoclast fusion from mononuclear precursors, and those of cellular adhesion, if blocked will lead to decreased resorption. As suggested earlier, transmembrane phospholipid trafficking by the ABC1 transporter provides a mechanism for osteoclast fusion, whilst cellular adhesion may be promoted by annexin-mediated binding between phospholipids and extracellular matrix.

The applicant has gone on to convincingly demonstrate that inhibition of ABC proteins using Glibenclamide, a known inhibitor of known ABC proteins, in osteoclast containing populations inhibits resorption. These results demonstrate that inhibitors or antagonists of ABC proteins will be useful therapeutic agents in the therapy of diseases where inhibition of resorption is desirable. These include osteopenia, which includes osteoporosis, Paget's disease, bone metastases, myeloma periodontal disease and humoral hypercalcaemia of malignancy.

Additionally, the applicant has surprisingly found that parathyroid hormone (PTH), a known stimulant of bone resorption, when present with compounds of the present invention, enhances the inhibitory effect of the compound.

According to a first aspect of the present invention there is provided a compound which will act as an inhibitor or antagonist of an ABC protein in bone for use in the manufacture of a medicament for use in the treatment of a disease where full or partial inhibition of bone resorption will result in an improvement in the disease.

Examples of existing compounds which have similar action to the sulphonyl urea, Glibenclamide, include:

TOLBUTAMIDE,

CHLOROPROPAMIDE,

TOLOZAMIDE,

GLIPIZIDE,

GLIQUIDONE, and

GLICLAZIDE.

The compounds may be administered orally, intravenously, subcutaneously or by any other traditional route. For oral application, the compounds of the present invention can be administered in such oral dosage forms as tablets, capsules (each of which includes sustained release or timed release formulations), pills, powders, granules, elixirs, tinctures, suspensions, syrups and emulsions.

The dosage regimen utilising the compounds of the present invention is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; and the particular compound employed. An ordinarily

skilled physician or veterinarian can readily determine and prescribe the effective amount of the compound required to prevent, counter or arrest the progress of the condition

The adult dosage may, for oral application, range from 0.001 to 5g daily, more preferably 0.01g to 0.5g daily.

The preferred daily dose is from 0.000014g to 0.0715g per kg of body weight, and more preferably from 0.00014g to 0.00715g. Thus for a 70kg adult the daily dose can range from 0.001g to 5.0g, more preferably from 0.01 to 0.5g. This daily dose may be administered in divided doses from 1 to 4 times a day giving unit doses for an adult of from 0.00025 to 5.0g.

The compounds of the present invention can form the active ingredient, and are typically administered in admixture with suitable pharmaceutical diluents, excipients or carriers suitably selected with respect to the intended form of administration.

For instance, for oral administration in the form of a tablet or capsule, the active drug component can be combined with an oral, non-toxic, pharmaceutically acceptable, inert carrier such as lactose, starch, sucrose, glucose, methyl cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, mannitol, sorbitol and the like; for oral administration in liquid form, the oral drug components can be combined with any oral, non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents and coloring agents can also be incorporated

in to the mixture. Suitable binders include starch, gelatin, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum and the like.

The compounds may be administered by any means that treat and/or prevent conditions where full or partial inhibition of bone resorption is desirable. Such conditions include osteopenia, which includes osteoporosis, Paget's disease, bone metastases, myeloma, perodontal disease and humeral hypercalcaemia or malignancy.

Compounds of the present invention may be useful for treating and/or preventing conditions prevalent in post-menopausal women; in particular those individuals suffering from osteoporosis.

Furthermore, the compounds of the present invention may be useful for treating and/or preventing conditions prevalent in Caucasian elderly men (e.g. individuals over 50 years of age); in particular, those individuals suffering from Paget's disease.

In addition, the compounds of the present invention may be useful in preventing accelerated bone loss in individuals who are genetically disposed to suffer from disease where loss of bone occurs.

According to another aspect of the present invention there is provided a

composition which will act as an inhibitor or antagonist of an ABC protein for use in the manufacture of a medicament for use in the treatment of a disease where full or partial inhibition of bone resorption will result in an improvement in the disease comprising a compound as described hereinabove and parathyroid hormone (PTH).

The compounds may be administered concomitantly with PTH. Alternatively, PTH may be administered separately. The mode of administration may be the same or different as that for the compounds of the present invention.

The dosage of PTH may vary according to the criterion set out for the compounds of the present invention, as hereinabove described.

The adult dosage may, for oral application, range from 1 to 200 International Units daily, more preferably 50 to 100 International Units.

The preferred daily dose of PTH, is from 0.014 to 2.9 International Units per kg of body weight and more preferably from 0.7 to 1.4 International Units. Thus for a 70 kg adult, the daily dose can range from 1 to 200 International Units, more preferably 50-100 International Units. This daily dose may be administered in divided doses from 1 to 4 times a day giving unit doses for an adult of from 12.5 to 200 International Units.

Animals which may be treated according to the methods of the present invention include all animals which may benefit therefrom. Included in such animals are humans and horses, although the invention is not intended to be so limited.

This discovery also allows candidate compounds to be screened.

According to a further aspect of the present invention there is provided a

method of screening for a compound which will lead to full or partial inhibition of bone resorption, which comprises determining whether the compound acts as an inhibitor or antagonist of the expression or function of an ABC protein in bone.

According to a further aspect of the present invention there is provided a method of screening for a compound which will act as an inhibitor or antagonist of the expression or function of an ABC protein in bone comprising determining whether the presence of said compound leads to full or partial inhibition of bone resorption.

The method may comprise the use of osteoclasts and/or osteoclast precursors. Osteoclasts may be obtained from any suitable source. Preferably, human bone, human bone marrow, human blood or any suitable tissues from experimental animals. More preferably, osteoclast precursors are obtained from human blood.

According to a further aspect of the present invention there is provided a method of treatment of a disease where full or partial inhibition of bone resorption will result in an improvement in the disease comprising administering a compound which will act as an inhibitor or antagonist of an ABC protein in bone.

The invention will be further described, by way of example only, with reference to the following test data.

Identification of novel ABC transporters from human giant cell tumour

Monoclonal antibodies were raised against cells from human bone. One antibody was shown to strongly stain the osteoclast. In order to identify the antigen

a human bone cDNA expression library constructed in lambda gt11 was immunoscreened. Two clones were identified of size 300bp and 435bp. A second separate immunoscreen identified the 435bp clone which was then sequenced and identified as a partial length cDNA clone encoding a novel ATP binding cassette (ABC) protein. Subsequently this clone was used to hybridization screen an osteoclastoma cDNA library in lambda gt11 and 19 clones were identified and purified. The longest sequenced was 1.5kb and found to be a highly homologous, but distinct from the 435bp clone and encoded a second novel ABC transporter. Further sequencing of the additional clones suggests that there may be additional family members. Comparative sequence analysis and phylogenetic analysis demonstrates that these ABC's comprise a novel sub family of transporters which have not previously been described. This novel sub family of ABC transporters would appear to have restricted tissue expression.

Inhibition of resorption by Glibenclamide.

In order to determine whether ABC proteins were involved in bone resorption the applicant conducted in vitro bone resorption assays (as per C.A. Walsh et al, Journal of Bone and Mineral Research, volume 6, number 7, 1991 with avian osteoclasts, and Methods in Molecular Medicine, Human cell culture protocols, ed G.E. Jones, Humana Press, pages 263- 275 with human osteoclasts) to look at the effect of a well characterised ABC protein inhibitor, Glibenclamide, using avian and human osteoclasts. Active factors known to have an effect on bone resorption and /or Glibenclamide were also introduced into the assays. The active factors included

parathyroid hormone (PTH), which is known to stimulate bone resorption in this system, and ATP since it has been suggested that Glibenclamide inhibits ATP release from cells (E.M. Schwiebert et al, Cell, Vol 81, 1063-1073, 1995).

The results are shown in Figs. 1 to 3. Fig 1 shows inhibition of resorption by Glibenclamide, enhanced inhibition of resorption by Glibenclamide and PTH, and inhibition of resorption by Glibenclamide in the presence of ATP, using avian osteoclasts in vitro according to the above test;

Fig.2 shows inhibition of resorption by Glibenclamide using human osteoclasts derived from a giant cell tumor according to the above test;

Fig. 3 shows the dose response as inhibition of resorption by Glibenclamide using human osteoclasts according to the above test.

The vehicle is tissue culture medium containing 0.1% dimethyl sulphoxide.

In summary, Glibenclamide at concentrations of 100 micromolar inhibited resorption by settled suspensions of avian bone cells (Fig.1). Inhibition of resorption was enhanced by PTH in the presence of Glibenclamide (Fig.1). In addition, exogenous ATP does not overcome resorption inhibition by Glibenclamide (Fig.1).

These findings were supported using a suspension of giant cells obtained from a human osteoclastoma or giant cell tumour (Fig.2). Glibenclamide at concentrations lower than 50 micromolar did not inhibit resorption, however, at concentrations of 50 and 100 micromolar resorption was depressed (Fig.3). This was confirmed using a different giant cell population. This data demonstrates that inhibiting ABC proteins inhibits bone resorption.

More details of the procedure followed are given below.

Avian osteoclasts were isolated from the femora and tibiae of pre-hatch chicks and seeded on to sterile devitalised dentine wafer. Cells were allowed to settle for 24 hours after which time the wafers were washed to remove non-adherent cells. Fresh medium containing active factors (PTH, ATP and/or Glibenclamide) were added for 72 hours. At the end of this period the wafers were fixed.

Dentine wafers were then washed in PBS at 37°C, fixed in 4% glutaraldehyde in 0.2% sodium cacodylate, and stained for 5 mins in 1% (w/v) toluidine blue in 0.5% disodium tetraborate. Resorption lacunae present on stained devitalised bone wafers were identified using an Olympus BH2 microscope fitted for incident light microscopy with metallurgic objectives. The plan area of resorption was determined by point counting using a 10X objective and drawing tube and expressed as a percentage of the total plan area of the bone wafers.

Human osteoclasts were dislodged from human giant cell tumour (GCT) by agitation in αMEM. Sterile devitalised dentine wafers were placed in a culture dish and the GCT suspension dripped over them using a sterile 10ml syringe. The culture was then incubated at 37 °C in a humidified atmosphere of 95% air and 5% CO_2 for 20 min. Wafers were then removed and washed in PBS to dislodge any non-adherent cells. Wafers were then transferred to 24 well plates, each containing 900µl of αMEM supplemented with 10% foetal calf serum and incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2 for 24 hours. Cells were treated by adding 100µl of 10x concentration of inhibitor and incubated at 37°C for a further

72 hours as described above. Dentine wafers were then washed, fixed, stained and viewed as detailed in the avian osteoclast procedure.

CLAIMS

1. A compound which will act as an inhibitor or antagonist of an ABC protein in bone for use in the manufacture of a medicament for use in the treatment of a disease where full or partial inhibition of bone resorption will result in an improvement in the disease.

2. A compound as claimed in claim 1 selected from the group consisting of glibenclamide, tolbutamide, chlorpropamide, tolozamide, glipizide, gliquidone and gliclazide.

3. A compound as claimed in claim 1 or 2, wherein the compound is in a form for oral or intravenous administration.

4. A compound as claimed in claim 1, 2 or 3 which is in a unit dosage form.

5. A compound as claimed in claim 4 wherein the compound is present in unit dosage form in an amount of from 0.001g to 5g.

6. A compound as claimed in claim 4 wherein the compound is present in unit dosage form in an amount of 0.01g to 0.5g daily.

7. A compound as claimed in claim 4 wherein the compound is present in unit dosage form in an amount of from 0.000014g to 0.0715g per kg of body weight daily.

8. A compound as claimed in claim 4 wherein the compound is present in unit dosage form in an amount of 0.00014g to 0.00715g per kg of body weight daily.

9. A compound as claimed in any of claims 1 to 8 for use in the manufacture

of a medicament for use in the treatment of osteoporosis.

10. A composition which will act as an inhibitor or antagonist of an ABC protein in bone for use in the manufacture of a medicament for use in the treatment of a disease where full or partial bone resorption will result in an improvement in the disease comprising a compound as claimed in any preceding claim and parathyroid hormone.

11. A composition as claimed in claim 10 wherein parathyroid hormone is in a form for oral, subcutaneously or intravenous administration.

12. A composition as claimed in claim 10 or 11 which is in a unit dosage form.

13. A composition as claimed in claim 12 wherein parathyroid hormone is present in unit dosage form of from 1 - 200 International Units.

14. A composition as claimed in claim 12 wherein parathyroid hormone is present in unit dosage form of from 50 - 100 International Units.

15. A composition as claimed in claim 12 wherein parathyroid hormone is present in unit dosage form of from 0.014 to 2.9 International Units per kg of body weight.

16. A composition as claimed in claim 12 wherein parathyroid hormone is present in unit dosage form in an amount from 0.7 to 1.4 International Units per kg of body weight.

17. A method of screening for a compound which will act as an inhibitor or antagonist of the expression or function of an ABC protein in bone comprising

determining whether the presence of said compound leads to full or partial inhibition of bone resorption.

18. A method of screening for a compound which will lead to full or partial inhibition of bone resorption, which comprises determining whether the compound acts as an inhibitor or antagonist of the expression or function of an ABC protein in bone.

19. A method of treatment of a disease where full or partial inhibition of bone resorption will result in an improvement in the disease comprising administering a compound which will act as an inhibitor or antagonist of an ABC protein in bone.

20. A method of treatment of a disease where full or partial inhibition of bone resorption will result in an improvement in the disease comprising administering a composition comprising a compound, which will act as an inhibitor or antagonist of an ABC protein in bone, and parathyroid hormone.

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(54) Title: THERAPEUTIC USE OF AN INHIBITOR OR AN ANTAGONIST OF AN ABC PROTEIN IN BONE

(57) Abstract: Use of a compound which will act as an inhibitor or antagonist of the expression or function of an ABC protein, and a method of screening for a compound which will act as an inhibitor or antagonist of the expression or function of an ABC protein.

1/2

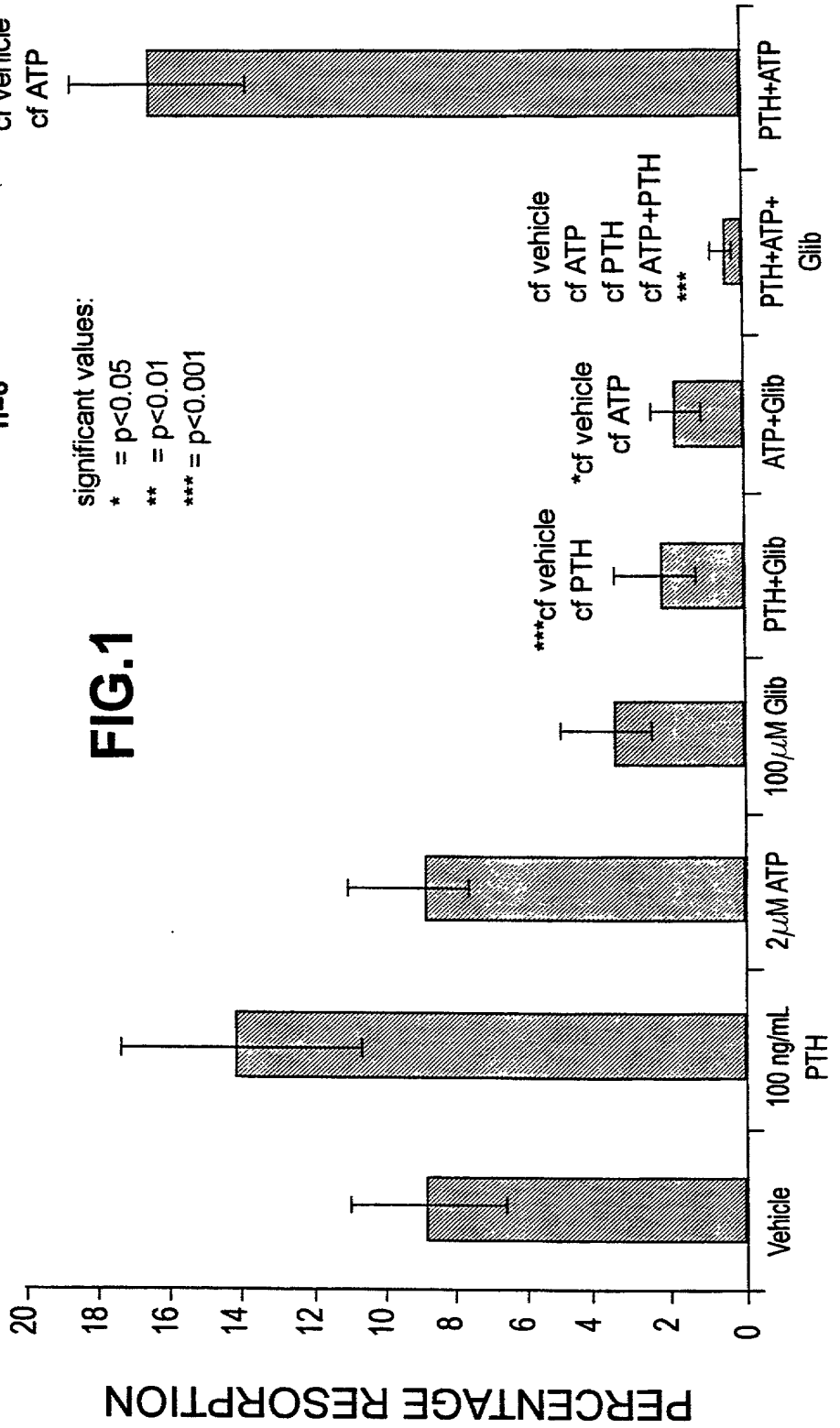
Avian,24hr settle,72hr inc,
n=6
significant values:
* = p<0.05
** = p<0.01
*** = p<0.001
** cf vehicle
cf ATP
***cf vehicle
cf PTH
*cf vehicle
cf ATP
cf vehicle
cf ATP
cf PTH
cf ATP+PTH
***
PERCENTAGE RESORPTION
0
2
4
6
8
10
12
14
16
18
20
Vehicle
100 ng/mL
PTH
2μM ATP
100μM Glib
PTH+Glib
ATP+Glib
PTH+ATP+
Glib
PTH+ATP
TREATMENTS

FIG.1

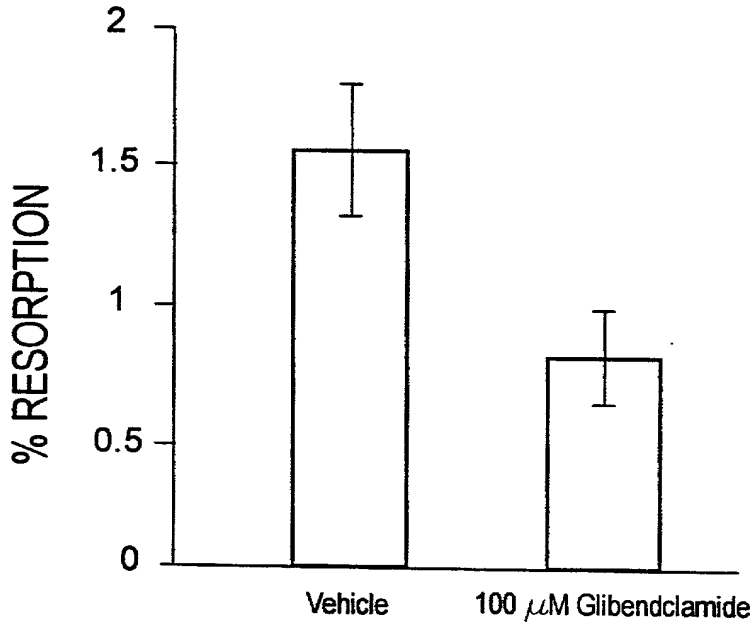

FIG.2.

n=7

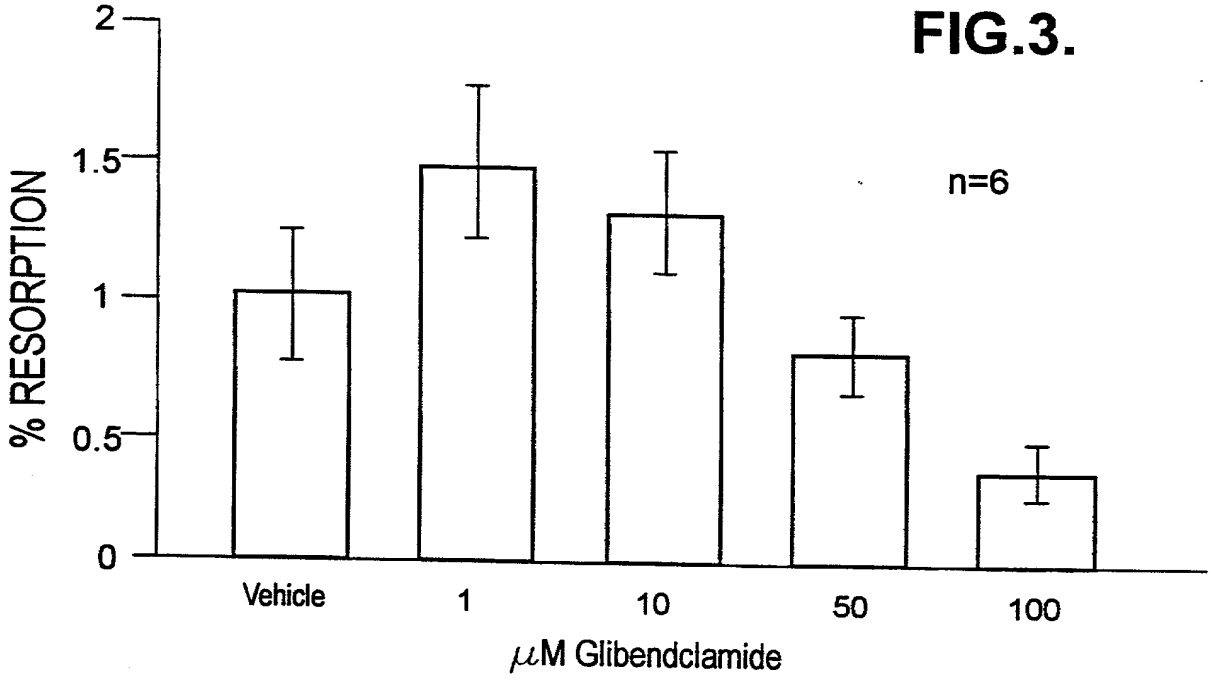

FIG.3.

n=6

PATENT
Docket No. 303212000600

DECLARATION FOR UTILITY PATENT APPLICATION

AS A BELOW-NAMED INVENTOR, I HEREBY DECLARE THAT:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled: A COMPOUND FOR USE IN MEDICINE, the specification of which is attached hereto unless the following box is checked:

☒ was filed on November 7, 2001 as United States Application Serial No. 09/980,717 and on May 5, 2000 as PCT International Application No. PCT/GB00/01736.

I HEREBY STATE THAT I HAVE REVIEWED AND UNDERSTAND THE CONTENTS OF THE ABOVE-IDENTIFIED SPECIFICATION, INCLUDING THE CLAIMS, AS AMENDED BY ANY AMENDMENT REFERRED TO ABOVE.

I acknowledge the duty to disclose information which is material to the patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

Application No.	Country	Date of Filing (day/month/year)	Priority Claimed?
PCT/GB00/01736	PCT	5 May 2001	☒Yes ☐No
9910693.2	GB	7 May 1999	

I hereby claim benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

Application Serial No.	Filing Date

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to

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Application Serial No.	Filing Date	Status
		☐Patented ☐Pending ☐Abandoned

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00

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